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(54) Title: PEPTIDE REPRESENTING ANTIGENIC EPITOPES OF FELINE IGE PRESENT ON FELINE B-CELLS BUT NOT FELINE BASOPHIL CELL SURFACES

(57) Abstract

Antigenic epitopes associated with the extracellular segment of the domain which anchors immunoglobulins to the feline B cell membrane are disclosed. For IgE, the epitopes are present on IgE-bearing B cells but not basophils or the secreted, soluble form of IgE. The epitope can be exploited for therapy and diagnosis. For example, peptides representing the epitopes associated with the anchor domain of IgE can be used to generate antibodies against these regions, for use in cat allergy therapy and analysis of cat IgE-bearing B lymphocytes.

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PEPTIDE REPRESENTING ANTIGENIC EPITOPES OF FELINE IGE PRESENT ON FELINE B-CELLS BUT NOT FELINE BASOPHIL CELL SURFACES

Background

The immediate-type hypersensitivities, such as extrinsic asthma, hay fever, and allergic responses to certain food or drugs, are mediated primarily by immunoglobulin E (IgE). In an IgE-mediated allergic response, the allergen binds to IgE on the surface of mast cells and basophilic leukocytes (basophils). This binding causes a crosslinking of the IgE molecules and hence, the underlying receptors for the Fc portion of IgE (FcϵR), and thereby triggers the release of pharmacologic mediators, such as histamine, the slow-reacting substance of anaphylaxis, and serotonin. The release of these mast cell and basophil products causes the various pathological manifestations of allergy.

IgE is produced by a particular class of B lympphocytes (B cells), which also bear IgE on their surface. In individuals sensitized to specific allergens, the allergen-specific IgE is produced by B cells continuously.

IgE binds to the receptors for the Fc of IgE (FcϵR) on the surface of basophils and mast cells very strongly. The association constant, Ka, is in the neighborhood of 1 x 10^{10} liter/mole and the "off" time is more than 20 hour. The very strong and stable association of IgE with FcϵR means that IgE is virtually always present on these cells. An immunotherapeutic agent targeting the IgE on B cells must not react with

the IgE on basophils and mast cells. Antibodies which react with the IgE isotype will cross-link IgE and the underlying FcϵR on basophils and mast cells and, when administered *in vivo*, will induce systemic release of pharmacologic mediators, leading to anaphylaxis.

The development of monoclonal antibodies that recognize an antigenic epitope present on the IgE on B cells, but not the IgE on basophils, was described in International Application Serial No. PCT/US88/04706. These antibodies also bind to the secreted form of IgE. In addition, the method of using such antibodies for treating allergy, either in the form of plain antibodies or toxin-conjugated antibodies, was described. The plain antibodies can cause the pharmacological mechanism of antibody-dependent cellular cytotoxicity (ADCC), and the toxin-conjugated antibodies can directly cause cytolysis. Both of these mechanisms can lead to the depletion of IgE-bearing B cells without harming the basophils and mast cells. The IgE epitopes present on B cells but absent on basophils are termed ige.bl epitopes (bl stands for B lymphocytes).

If a vaccine was developed which induced production of antibodies which could specifically target IgE-bearing B cells, without targeting any other cells, such a vaccine would be useful in treating allergy and related hypersensitivities. It has been observed that IgE is not needed for normal

health, except perhaps for combatting parasites. Thus, depleting all IgE-bearing B cells would not produce any adverse effects.

Summary of the Invention

The invention relates to antigenic epitopes (and peptides representing these epitopes) which are present on feline B cell membrane-bound immunoglobulins but not on the secreted, soluble form of the immunoglobulin. The invention also pertains to methods of exploiting these newly discovered epitopes for therapeutic and diagnostic purposes.

B cells express on their surface antibody molecules, which serve as receptors for antigens during immunological induction. The membrane-bound immunoglobulins differ from the secretory, soluble immunoglobulins synthesized by the same cells in that they have an extra peptidic piece that anchors them onto the B cell surface.

All ten membrane-bound immunoglobulins on B cells from different species, for which amino acid sequences have been determined, have extra isotype-specific regions that anchor the immunoglobulins to the membrane. These peptidic regions have lengths ranging from 41 to 72 amino acids, and can be divided into three segments in terms of locations in relation to the plasma membrane. The middle segments of 25 hydrophobic and uncharged amino acid residues are in the membrane lipid bilayer. The C terminal hydrophilic segments of 3-28 amino acid residues are intracellular. The segments toward N-terminus contain 13 to 27 amino acid residues and are highly acidic and hydrophilic. They are located on the extracellular surface of the plasma membrane.

The length and the hydrophilic and highly charged nature of the extracellular segment indicates that this segment is exposed and accessible to antibodies. These antigenic epitopes located on the extracellular segment of membrane-bound region of immunoglobulin heavy chains can be generally designated mb/ec, and the mb/ec segment of IgE is designated as the ϵ.mb/ec segment.

The present invention pertains to the discovery of feline ϵ.mb/ec epitopes and peptides representing them, and the use of such peptides for feline allergy treatment and for generating monoclonal and polyclonal antibodies which target the feline ϵ.mb/ec epitopes on B cells.

Detailed Description of the Invention

1. Unique Antigenic Epitopes of IgE as Targets of Immunotherapeutic Agents

IgE is present on three cell types in the body: IgE-producing B cells, mast cells, and basophils. If an antigenic epitope of IgE is present on B cells and not on basophils and mast cells, these epitopes (defined as ige.bl) are virtually unique cell surface markers of IgE-bearing B cells. These markers provide targets for several types of monoclonal or polyclonal antibody-based therapy for IgE-mediated allergic diseases, and provide a means to differentiate B cells producing IgE from B cells producing other isotypes.

2. Anchoring Peptidic Piece of B Cell Membrane-Bound Immunoglobulins

The amino acid sequence data of the ten membrane-bound immunoglobulins from several species have been determined. *See* Ishida, N. et al., EMBO J., 1:1117 (1982); Steen, M. L. et al., J. Mol. Biol., 177:19-32 (1984); Rogers, J. et al., Cell, 26:19-27 (1981); Yamawaki-Kataoka, Y. et al., Proc. Natl. Acad. Sci., USA, 79:2008-2012 (1982); Kamaromy, M. et al., Nuc. Acids Res., 11:6775-6785 (1983); Rogers, J. et al., Cell, 20:303-312 (1980); Bernstein, K. E., J. Immunol. 132:490-495 (1984); Cheng, H. et al., Nature, 296:410-415 (1982). These sequences indicate certain common features of the plasma membrane-bound peptidic piece. The peptidic anchor piece has three segments which are distinguishable based upon their locations in relation to the plasma membrane. Even though these peptidic pieces are short, ranging from 41 to 72 amino acid residues, and have often been referred to as the "membrane-bound domain", the peptides are not entirely in the membrane lipid bilayer. In fact, only 25 amino acid residues (which are the largely hydrophobic residues and threonine and serine) located in the middle part of the peptides, are in the lipid bilayer. The C-terminal, hydrophilic segments of 3 to 28 amino acid residues are located on the cytoplasmic side of the membrane. The segments toward the N-terminus, which are connected to the third or fourth constant domains of the immunoglobulin heavy chains (CH_3 or CH_4) are very hydrophilic and are on the extracellular side of the plasma membrane.

The shortest of the extracellular segments of the membrane-bound pieces of the immunoglobulins (which are the mouse and rabbit μ chains) have 13 amino acid residues. The mb/ec segments of all immunoglobulins contain high proportions of charged amino acid residues, and are almost entirely acidic residues. The proportions of charged amino acid residues and polar hydrophilic residues account for very high percentages of the amino acid composition of the mb/ec segment. These parameters indicate that all the mb/ec segments are exposed and long enough to be accessible by antibodies. Studies of the evolution of immunoglobulin heavy chains indicate the ϵ and γ chains are more related to each other (had more recent common ancestry) than to other chains (Lin, L. C. and Putnam, F. W., Proc. Natl. Acad. Sci. USA, 1981). In addition, the heavy chains evolved before the various mammals species, such as mice, rats, and humans, evolved. The murine or rat ϵ.mb/ec segment has 19 amino acid residues, among them 8 Glu and 2 Asp residues.

3. Determining the Amino Acid Sequence of the Human ϵ.mb/ec Segment.

A number of well established procedures can be applied to determine the DNA sequence corresponding to the human ϵ.mb/ec segment. In one approach, the starting point is the mRNA preparation of a human myeloma cell line which expresses IgE on the surface, for example, SK007 cells. With the mRNA preparation, one can establish a cDNA library employing cloning vectors with λ phage or plasmids. A

preferred method for constructing the cDNA library is with the cDNA Library Construction System Kit - Librarian I; developed and commercialized by Invitrogen (San Diego, CA). A stepwise detailed instruction manual is provided for RNA isolation from cells, reverse transcription, second strand synthesis, linker ligation, agarose gel sizing of cDNA, electroelution to purify cDNA vector ligation, and transformation of E. coli. The vector used in this library is pDCM8.

In the screening of the cDNA library for clones containing the ε.mb/ec segment, several probes can be used. The library can be screened with a DNA probe a, which is a 1.1 kb long U266 cDNA covering most of length of ε mRNA (no membrane-bound secreted and membrane-bound forms can be distinguished by using additional probes). A probe b is developed by taking advantage of the likelihood that the end of the CH_4 domain is truncated in the human ε chain of the membrane-bound form. The truncation occurs where gene segments of CH_4 domain and the membrane-bound domain are translocated. The loss of C-termini occur with the membrane-bound forms of immunoglobulins, including ε and λ, which contain CH_4 domains. From the published information on the nucleotide sequence of the human ε CH_4 domain, the most probable splicing donor site is intracodon GT, 71 bp 5' of the termination codon TGA. Another GT, which is not intracodon and less likely a splicing donor site, is closer to the terminus (24 bp 5' to the termination codon).

Probe b will react with secreted form of ϵ chain gene and not the membrane-bound form of the ϵ chain gene.

The design of a probe c is based on the finding that the transmembrane segment of the membrane-bound domain (mb/tm segment) is very conserved among all the immunoglobulin genes so far sequenced. There is a segment of peptide and its corresponding coding DNA within this mb/tm segment that is nearly identical among all immunoglobulins.

A probe d, which represents a segment upstream to the most possible splicing donor site, GT, consists of 36 bp. This probe should react with the ϵ chain gene of both the secreted and membrane-bound forms. The location of probes a, b, c, and d, in schematic form, is shown in U.S. Patent No. 5,091,313.

Table 1 summarizes the pattern of reactivities of clones containing ϵ genes of secreted or membrane-bound immunoglobulins with the four probes.

Table 1. The reactivity of ϵ gene-containing cDNA clones with probes a, b, c, and d.

	ϵ Secreted	ϵ Membrane-bound
Probe a	+	+
Probe b	+	-
Probe c	-	+
Probe d	+	+

The library size needed to clone the membrane-bound ϵ chain depends on how abundant the mRNA is. Assuming secreted IgE comprises 0.1% of the SK007 poly A^+ RNA, the library size should be about 5,000 independent recombinant clones, in order to have a 99% possibility of isolating a positive clone. In IgE-producing rat immunocytoma IR2 and IR162 cells, mRNA for the membrane-bound form of ϵ chain was found to be more than 2% of that of the secreted form. Assuming this ratio of membrane-bound/secreted forms of ϵ chain holds true for the human IgE-producing SK007 cells, the cDNA library size needed to isolate the membrane-bound ϵ chain is about 250,000. In a preferred procedure, a larger number of clones, about 1,000,000, are screened.

An alternative to the conventional approach of establishing a cDNA library and screening the clones representing the cellular mRNA species, is to amplify the mRNA to produce high proportions of their corresponding DNA. The resulting DNA can then be purified by gel

electrophoresis and subjected to sequence analysis. The methodology, referred to as polymerase chain reaction (PCR) amplification, has been established in the past few years and a complete system, including reagents and equipment, have been commercialized. One preferred system is provided by Perkin Elmer Cetus (Norwalk, CT). The reagent kit is the GeneAmp DNA Amplification Reagent Kit and the equipment is the DNA Thermal Cycler.

Some of the specific reagents used in this approach are the same as used for the cDNA library cloning. Since no sequence in the membrane-bound segment of the ϵ chain has been determined, the strategy is to amplify both the secreted and membrane-bound forms of the ϵ chains. Two primers are to be used, one is oligo.dT (25-30-mers) and one is the oligomer corresponding to probe d. Probe d is located 5' to the most possible splicing donor site and therefore primes both the secreted and membrane-bound forms of ϵ mRNA and DNA. After sufficient amplification, the two populations of DNA fragments are resolved by gel electrophoresis. The secreted form of the ϵ chain can be distinguished by its reactivity with probe b. The purified DNA is then subjected to DNA sequencing.

PCR amplification seems to be a more efficient procedure than cDNA cloning for mRNA poorly represented in the poly A^+ RNA pool.

The U266 ϵ chain cDNA can be used to work out some preliminary annealing conditions between template DNA and the oligo-primers.

Another approach for obtaining a DNA clone containing genes encoding the membrane-bound segments is to screen the human genomic DNA library. The human genomic DNA library is readily available. A preferred source is the library constructed using human lung fibroblast WI38 cells provided by Stratagene (La Jolla, CA). The genes are in λ vector and the inserted DNA have average sizes of 15K bp. Identification of the clones can be achieved by hybridization with U266 cDNA. The location of the gene segment corresponding to the membrane-bound region can be determined by using a probe prepared from the homologous mouse gene of the transmembrane segment (probe c, discussed above). The sequence of the membrane-bound segment is then determined, and this sequence is shown in U.S. Patent No. 5,091,313.

Nucleotide sequence of genomic DNA encompassing the encoding segments for the membrane anchoring peptide of human membrane bound ϵ chain was determined as described above. The assignment of the exons was made by identifying the nucleotides for splicing donors and acceptors, and by comparing them to the published homologous sequences of mouse membrane-bound ϵ chain, as well as to immunoglobulins of other classes. Two exons were found to exist.

Human <u>ε.mb/ec</u> peptide is identified as the first fourteen amino acids encoded by membrane exon I. This precedes a stretch of about 25 hydrophobic amino acids which are the transmembrane region. Two possible structures of <u>ε.mb/ec</u> are shown in U.S. Patent No. 5,091,313.

As described more fully below, the <u>ε.mb/ec</u> peptides (from both humans and other species) can be used to elicit antibodies which react specifically with membrane-bound immunoglobulin ε. For this purpose, the peptides can be chemically synthesized by standard techniques of protein synthesis. A preferred method for synthesizing the peptides is with the RaMPS system (DuPont, Wilmington, DE), which applies Fmoc chemistry. Alternatively, the proteins can be biosynthesized by employing oligodeoxynucleotides encoding the peptides.

As immunogens or vaccines, the peptides may be used in either the monomeric or dimeric structural forms shown in Patent No. 5,091,313. Peptides comprising the human <u>ε.mb/ec</u> segment and the connecting four amino acids in the CH_4 domain can also be used. In addition, modified peptides having substantial immunological equivalency can be used. For example, the peptide amino acid sequence shown above (or described below for dogs) can be modified by deletion, insertion or substitution of one or more amino acids which do not essentially detract from the immunological properties of the peptide. The peptides can also be used

as polymers where the amino acid sequence shown above, or equivalent sequence, is the polymer repeat unit.

4. Sequencing the Cat ϵ.mb/ec Segment.

A total of 5 x 10^5 recombinant bacteriophage plaques from a feline genomic library (purchased from Stratagene, Inc.) were screened by using a ^{32}P-labeled dog Cϵ cDNA probe. One phage clone was shown to contain sequences homologous to both the constant region and membrane domain of the dog IgE. *(See* U.S. Application Serial No. 08/031,398 (incorporated by reference) for these dog sequences.). A 1.6kb NcoI/HpaI DNA fragment containing the membrane domain was subcloned into a Bluescript cloning vector and the plasmid DNA was used for nucleotide sequencing. Two exons with homology to dog IgE membrane exons m1 and m2, respectively, were identified within this plasmid subclone. The DNA sequence of the cat m1 exon, together with its translated amino acid sequence, is shown in SEQ ID NO:1.

The cat *migis-ϵ* sequence is tentatively defined as the first 19 amino acid residues of the m1 region based on the hydrophilicity analysis. Comparison of the *migis-ϵ* peptide sequence between dog and cat showed that, in addition to an amino acid change at position 15 (Tyr in the cat and Glu in the dog), there is a homologous amino acid replacement at position 6 (Asp in the cat and Glu in the dog), and at position 12 (Ala in the cat and Thr in the dog).

6. Developing Antibodies to the ϵ.mb/ec Segment.

The ϵ.mb/ec peptide can be used in the immunization of animals to prepare polyclonal and monoclonal antibodies. They can also be used to screen for specific monoclonal antibodies or characterize specific polyclonal antibodies. They can also be used to purify monoclonal and polyclonal antibodies.

In the process of preparing for monoclonal antibodies specific for ϵ.mb/ec peptide, it is not necessary to use the ϵ.mb/ec peptide in both immunization and antibody identification. For example, in immunizing mice for preparing immune spleen cells for fusion with myeloma cells, the immunogen may be the membrane-bound IgE isolated from plasma membrane of IgE-bearing myeloma cells, such as SK007 cells. The immunogen may also be the IgE-bearing myeloma cells themselves. The immunogen can also be the purified dog IgE described above.

For using the synthetic ϵ.mb/ec peptide as the immunogen, it is more effective to conjugate the peptide to a protein carrier. A preferred protein carrier is keyhole limpet hemocyanin (KLH). If the peptidic segment lacks a lysine residue or if the lysine residue is in the middle part of the segment, it is desirable to add a lysine residue at the C-terminal end. Because the N-terminus already has an α-amino group, the modified synthetic peptidic will have two amino groups for linking.

Multiple molecules of peptides can be conjugated to each molecule of the carrier protein. With KLH, a preferred molar ratio for peptide/KLH is 10. The method of conjugation is very well established. Cross-linkers such as glutaraldehyde or bis (sulfosuccinimidyl) suberate or disulfosuccinimidyl tartarate (Catalogue #21579, 20591, Pierce Chemical Co., Rockford, IL) have been used. A preferred cross-linker is the latter.

The immunogen, such as the KLH conjugate, can be used to immunize rabbits, goats, rats, or mice to prepare polyclonal antibodies specific for the ε.mb/ec peptide. Lympocytes from the spleen or lymph nodes of immune mice and rats can also be taken to prepare hybridomas secreting monoclonal antibodies specific for the ε.mb/ec peptide. A preferred protocol to prepare the monoclonal antibodies is to fuse immune spleen cells of mice with non-secreting mouse myeloma cells, such as NS-1 or SP2/0 cells using polyethylene glycol.

In one immunization protocol for mice, 50 μg of the peptide-KLH conjugate in complete Fruend's adjuvant is injected subcutaneously into each mouse for priming. Two and four weeks later, the same amounts of antigen are given s.c. in incomplete Freund's adjuvant. At about the six week time point, the fourth antigen injection is given i.p. in saline. Mice are sacrificed 4 days after the last injection and the spleens are taken for preparing single cell suspension for fusion with myeloma cells. A similar

protocol can also be used for immunization with purified native human membrane-bound IgE (having attached membrane anchor domain) isolated from the plasma membrane of IgE-bearing human myeloma cells, such as SK007 cells. When human IgE-bearing cells are used as the immunogen, 1×10^7 cells are injected i.p. at two week intervals.

The fusion procedure with polyethylene glycol and other various procedures concerning cloning and hybridoma culturing have been well established, and the preferred protocol is the same as described by Hudson, L. and Hay. F.C., (Practical Immunology, 2nd edition, pp. 303-313, Blackwell Publishing Co., Boston). The screening of hybridomas for monoclonal antibodies, or the identification of polyclonal antibodies or the identification of polyclonal antibodies reactive with ϵ.mb/ec peptide can be performed with enzyme linked immunosorbent assays (ELISAs), using the synthetic ϵ.mb/ec peptide as the solid phase antigen. An alternative solid phase antigen is the conjugate of ϵ.mb/ec peptide with a different carrier protein such as bovine serum albumin different from that used in the immunogen. Further characteristics of the monoclonal and polyclonal antibodies would be that they bind to an ϵ.mb/ec peptide, or to IgE-bearing B cells, but do not bind to soluble IgE, or to IgE on basophils or mast cells (as measured by a histamine release assay).

7. Using the ϵ.mb/ec Peptides for Animal Treatment

The peptides of the invention can be tested in animal model systems to determine if they are suitable for allergy treatment in humans. Also, the peptides of the invention can be used to treat animals which are afflicted with allergies or other immediate-type hypersensitivities; for example, the cat ϵ.mb/ec peptide can be used to treat cat allergies or hypersensitivities. Similarly, the corresponding ϵ.mb/ec peptides from horses or other mammals, could be used to treat these animals.

When used to treat animals, the ϵ.mb/ec peptides, with a suitable carrier such as a recombinant protein antigen from rabies virus or from feline leukemia virus, are administered to the animal in a suitable pharmaceutical vehicle. Sub-cutaneous or intra-muscular injection is preferred as a route of administration. The dosage of peptide administered can vary widely depending on the size of the animal, but should be sufficient to create an immunogenic response in the animal. This would generally be in the range of about 10-100 μg/kg of animal body weight.

8. Animal Model Experiments.

One could also test the use of ϵ.mb/ec peptides in vaccination in a mouse model system, as described below. Mice are not known to develop allergic symptoms naturally. However, for demonstrating the pharmacologic mechanisms of the intended therapy in regard to the depletion of IgE-bearing B cells and IgE, the mouse can serve as a model.

The ε.mb/ec segment of the mouse has already been sequenced. Ishida, N. et al., EMBO J. 1:1117-1123 (1982). The 19 amino acid residue peptide is shown in that publication.

The ε.mb/ec peptide is synthesized in several forms, including one that has extra Leu-Lys residues at the C-terminus. The peptide or its KLH conjugate could be used as the essential vaccine component, to immunize mice. One then examines whether the vaccine induces antibodies which target the IgE-bearing B cells, by examining the following questions:

(a) Does the total IgE in circulation decline?

(b) Does the number of IgE-bearing B cells decline?

(c) Does the density of IgE on the surface of basophils decline?

(d) Do IgM and IgG specific for ε.mb/ec peptide cause different effects? The purpose of this question is to address the effects of antibody dependent cellular cytotoxicity (ADCC) in the depletion of IgE-bearing B cells. IgG, not IgM, is known to mediate ADCC.

9. Diagnostic Uses for the Antibodies of the Invention.

Antibodies against ε.mb/ec epitopes can be used to identify and enumerate IgE-bearing lymphocytes in a fluid sample, such as serum.

Thus, the various antibodies which target the ϵ.mb/ec epitope of a particular species (*e.g.*, cats) can be used diagnostically in that species for such identification and enumeration of its IgE-bearing B lymphocytes.

For this purpose, antibodies can be used in standard assay formats for determining cell surface antigens. In general, the antibody is contacted with a sample of the leukocytes to be tested under conditions which allow the antibody to bind IgE-bearing cells in the sample. The cells are then examined for binding of antibody. This can be accomplished by conventional cell staining procedures. For example, a fluorescently labeled second antibody can be used to detect binding of the anti-IgE antibody.

Equivalents

The terms, expressions and examples herein are exemplary only and not limiting, and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) General Information:
(i) Applicant: Chang, Tse Wen
(ii) Title of Invention: PEPTIDES REPRESENTING ANTIGENIC EPITOPES OF FELINE IgE PRESENT ON B CELL BUT NOT BASOPHIL SURFACE
(iii) Number of Sequences: 1
(iv) Correspondence Address:
(A) Addressee: Tanox Biosystems, Inc.
(B) Street: 10301 Stella Link Rd.
(C) City: Houston
(D) State: Texas
(E) Country: USA
(F) Zip: 77025
(v) Computer Readable Form:
(A) Medium Type: Diskette, 3.5 inch
(B) Computer: IBM PS/2
(C) Operating System: DOS 3.30
(D) Software: Wordperfect 5.1
(vi) Current application data:
(A) Application Number:
(B) Filing Date:
(C) Classification:
(vii) Prior Application Data:
(A) Application Number: 08/164,910
(B) Filing Date: 12/09/1993
(viii) Attorney/Agent Information:
(A) Name: Mirabel, Eric P.
(B) Registration Number: 31,211
(C) Reference/Docket Number: TNX88-03EEE-PCT
(ix) Telecommunication Information:
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(2) Information for SEQ ID NO:1:
(i) Sequence Characteristics:
(A) Length: 135 nucleotides
(B) Type: nucleic acid
(C) Strandedness: double stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:1

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GAG CTG GAG CTC CAG GAC CTG TGT GCG GAC GCC GCT GAG 39
Glu Leu Glu Leu Gln Asp Leu Cys Ala Asp Ala Ala Glu
                    5                   10
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AGC TAC GAG CTG GAC GAG CTG TGG ACC AGC CTG CTG GTC 78
Ser Tyr Glu Leu Asp Glu Leu Trp Thr Ser Leu Leu Val
    15                  20                  25

TTC ATC ACC CTC TTC CTG CTC AGC GTG AGC TAC GGA GCC 117
Phe Ile Thr Leu Phe Leu Leu Ser Val Ser Tyr Gly Ala
            30                  35

GCC ATC ACC CTC TTC AAG 135
Ala Ile Thr Leu Phe Lys
40                  45
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CLAIMS

1. A peptide corresponding to the ϵ.mb/ec segment of a cat.

2. A peptide having the sequence of amino acid residue numbers 1 to 19 of SEQ ID NO:1.

3. An isolated oligonucleotide consisting of the sequence of nucleotide numbers 1 to 57 of SEQ ID NO:1, or the corresponding or complementary mRNA sequence.

4. A peptide having the sequence shown in SEQ ID NO:1.

5. An isolated oligonucleotide consisting of the sequence shown in SEQ ID NO:1, or the corresponding or complementary mRNA sequence.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14407

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07K 14/435, 7/00, 7/04; C07H 21/00
US CL :530/324, 326, 350; 536/23.1, 23.53
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 326, 350; 536/23.1, 23.53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

SEQUENCE SEARCH, APS, MEDLINE, BIOSYS, EMBASE, LIFESCI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,091,313 (CHANG) 25 FEBRUARY 1992, ENTIRE DOCUMENT, ESPECIALLY COLUMNS 3-9.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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